

TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371

1599-0213P

U.S. APPLICATION NO. (If known, see 37 CFR 1.5)

10/049663

INTERNATIONAL APPLICATION NO.

PCT/KR99/00460

INTERNATIONAL FILING DATE

August 18, 1999

PRIORITY DATE CLAIMED

TITLE OF INVENTION

IMMUNOLOGICAL TOLERANCE-INDUCTION AGENT

APPLICANT(S) FOR DO/EO/US

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CHO, Chul-Soo; PARK, Sung-Hwan; WKIM, Wan-Uk

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39 (1).
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
 - a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau). WO 01/12222
 - b. ☒ has been transmitted by the International Bureau.
 - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
 - a. ☐ is transmitted herewith.
 - b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4)
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).
 - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☐ have been transmitted by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 20. below concern document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98, Form PTO-1449(s), and International Search Report (PCT/ISA/210) with 0 cited document(s).
12. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment
14. ☐ A SECOND or SUBSEQUENT preliminary amendment
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821-1.825.
18. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4)
19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. ☒ Other items or information:
 - 1.) PCT/IB/308
 - 2.) PCT/IPEA/409
 - 3.) Twelve (12) sheets of Formal Drawings
 - 4.) Three (3) sheets of Sequence Listing

Page PTO-1390 (REV. 11-26-00) page 2 of 2

Rec'd PCT/PTO

22 MAY 2002

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#4

Application No. 10/049,663

BOX SEQUENCE
PATENT
1599-0213P

IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicant:	H.Y. KIM et al.	Conf.:	4710
Appl. No.:	10/049,663	Group:	1646
Filed:	February 15, 2002	Examiner:	TO BE ASSIGNED
For:	IMMUNOLOGICAL TOLERANCE-INDUCTION AGENT		

AMENDMENT

Assistant Commissioner for Patents
Washington, DC 20231

May 22, 2002

Sir:

In response to the Notification of Missing Requirements Under 35 U.S.C. 371 in the United States Designated/Elected Office (DO/EO/US) mailed April 16, 2002, the following amendments and remarks are respectfully submitted in connection with the above-identified application.

IN THE SPECIFICATION:

Please delete the Original Sequence Listing, pages following specification. Please insert the attached Substitute Sequence Listing, independently numbered 1-3, directly after the abstract.

REMARKS

Enclosed herewith in full compliance with 37 C.F.R. §§1.821-1.825 is a Substitute Sequence Listing to be inserted into the specification as indicated above. The Substitute Sequence Listing in no way introduces new matter into the specification. Also submitted herewith in full compliance with 37 C.F.R. §§1.821-1.825 is a disk copy of the Substitute

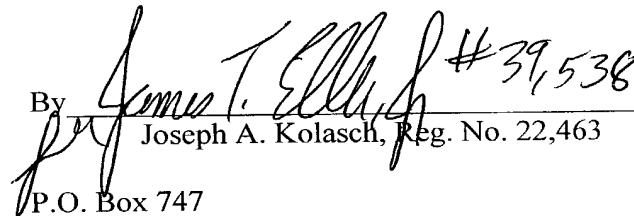
Application No. 10/049,663

Sequence Listing. The disk copy of the Substitute Sequence Listing, file "1599-0213P.st25.txt", is identical to the paper copy, except that it lacks formatting. No new matter is introduced by these amendments.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,
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By

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Attachments:

Disk Copy of Substitute Sequence Listing
Paper Copy of Substitute Sequence Listing
Copy of Notice

(Rev. 03/27/01)

JC11 Rec'd PCT/PTO 15 FEB 2002

PATENT
1599-0213P

IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicant: KIM, Ho-Youn et al.
Int'l. Appl. No.: PCT/KR99/00460
Appl. No.: New Group:
Filed: February 15, 2002 Examiner:
For: IMMUNOLOGICAL TOLERANCE-INDUCTION
AGENT

PRELIMINARY AMENDMENT**BOX PATENT APPLICATION**

Assistant Commissioner for Patents
Washington, DC 20231

February 15, 2002

Sir:

The following Preliminary Amendments and Remarks are respectfully submitted in connection with the above-identified application.

AMENDMENTS**IN THE SPECIFICATION:**

Please amend the specification as follows:

Before line 1, insert --This application is the national phase under 35 U.S.C. § 371 of PCT International Application No. PCT/KR99/00460 which has an International filing date of August 18, 1999, which designated the United States of America and was published in English.--

Docket No. 1599-0213P

REMARKS

The specification has been amended to provide a cross-reference to the previously filed International Application.

Entry of the above amendments is earnestly solicited. An early and favorable first action on the merits is earnestly solicited.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

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1599-0213P

(Rev 11/13/01)

12/Pt₂IMMUNOLOGICAL TOLERANCE-INDUCTION AGENT

FIELD OF THE INVENTION

1. FIELD OF THE INVENTION

The present invention relates to an immunological tolerance-induction agent.
5 More particularly, it relates to a use of biodegradable polymers as an immunological tolerance-induction agent and a method for treating autoimmune diseases in mammals.

2. DESCRIPTION OF THE PRIOR ARTS

Injection of a foreign substance (referred to as 'antigen') into a vertebrate
10 organism may result in the induction of an immune response characterized by the production of specific antibodies capable of interacting with the antigen or the development of effector T lymphocytes and the precaution of soluble mediators at the site of encounter with the antigen. Although antibodies and T lymphocytes do play an essential role in protecting against hostile antigen, they can also participate in
15 injurious processes, leading to destruction of host tissues. This is the case in autoimmune diseases where one's own antibodies and/or T lymphocytes react with antigen of one's own tissues and damage the tissues. This also is the case in allergic reactions characterized by an exaggerated immune response to certain environmental matters and which may result in inflammatory responses leading to tissue destruction.

20 An approach to treat effectively the diseases caused by unwanted or tissue damaging immunological reactions such as autoimmune diseases is to specifically suppress or decrease to an acceptable level the intensity of deleterious immune

processes without affecting the remainder of the immune system. Immunological tolerance deals with all mechanisms that ensure an absence of destructive immune response to any given foreign substance.

Orally administered autoantigens suppress autoimmunity in animal models, including experimental allergic encephalomyelitis (EAE), collagen-induced arthritis (CIA), uveitis, and diabetes in the non-obese diabetic mouse (1-4). Oral tolerance describes the observation that a state of hyporesponsiveness follows an immunization with a previously and repetitively fed protein. Recently, the antigen-specific tolerance induction has been utilized as a treatment for various autoimmune diseases. For example, the repetitive administration of type II collagen (CII) or major basic protein (MBP; myeline basic protein) has been reported to be effective for the treatment of CIA or EAE, respectively. Likewise, oral administration of S-antigen can suppress the development of experimental uveitis (1-4). Oral tolerance induction has advantages that it doesn't elicit side effects including leukopenia, increased susceptibility of infection or malignance which can be induced by other drugs used currently for treating autoimmune disease, since it can selectively inhibit pathologic immune responses rather than non-specific immune suppression. However, irrespective of apparent ideality of oral tolerance induction, only few reports described the effectiveness of the oral tolerance in human disease, and even the results are not entirely uniform.

Several factors may affect the effectiveness of tolerance induction. Nature of antigen (soluble or particulate antigens) (5), antigen dose (6,7), genetic background (8), role of digestive flora (9), and antigen uptake (10) are known to be important factors in the determination of tolerance effect. In particular, oral tolerance is associated with the presence of immunologically relevant antigen in circulation. The amount of antigen absorbed intactly varies widely and is extremely small, ranging from 0.001% to 1% of the administered dose, which is partly attributed to the intraluminal digestion before the absorption of antigen (10-12). In this respect, the

enhancement of the absorption rate of antigen administered orally seem to be promising in the tolerance therapy of human autoimmune disease.

Biodegradable microparticles have been studied as a drug carrier system, because of their advantages such as enhanced absorption, biocompatibility, sustained
5 drug release and so on (13-15). Particularly, since particles can slowly release any entrapping substances into surroundings for a long period, they can deliver several kinds of drugs, hormones, and vaccines for 1 to 3 months, even though they are administered only once. Recently, it has been reported that particulate antigens administered orally are absorbed well into Peyer's patches, which are the primary
10 immune system of the gastrointestinal tract (16-18). Furthermore, soluble antigens entrapped by particulates can be protected from acidic and enzymatically hostile environments found at mucosal surfaces, facilitating uptake of the antigens by M cells overlying Peyer's patches and mucosal epithelium, thereby promoting antigen transport to local lymphoid follicles and associated lymph node, and possibly
15 enabling the local accumulation of the antigen (19-22). Owing to these properties of particulate antigens, studies for particles are mainly focused on the vaccine delivery system in immunology.

Possible accumulation of particulate antigens in Peyer's patch and subsequent translocation to discrete anatomical compartments such as liver and spleen could
20 modulate both mucosal and systemic immune responses. Recently, it is documented that several kinds of polymer have a potential to suppress pathologic immune response. Copolymer 1, a synthetic amino acid copolymer composed of L-alanine, L-glutamic acid, L-lysine, and L-tyrosine, has a beneficial effect on the development of EAE that is associated with down-regulation of T cell immune responses to MBP,
25 an autoantigen of EAE, and is mediated by Th2/3 type regulatory cells (23). In CIA, copolymer 1 also competitively inhibits the interaction of CII with RA-associated HLA-DR molecules and thereby suppresses CII reactive T cell responses (24). Polyethylene glycol (PEG), another straight amphophilic polymer, is also proved to

Rheumatoid arthritis (RA) is a representative autoimmune disease in human and characterized by destructive polyarthritis. Although the etiologic agent of RA remains unclear, CII is a strong candidate because of its abundance in cartilage, an immunologically privileged tissue, and because of its ability to induce destructive immune-mediate polyarthritis after immunization of susceptible rodents and higher primates (27,28). Lymphocytes responding to CII are identified in the peripheral blood, or in the joints of patients with RA (29), and antibodies to CII are also evident in the sera, synovial fluid (SF), and cartilage in patients with RA (30,31), suggesting CII may act as an autoantigen in human RA.

Antigen-specific immune suppression using CII has been tested in human RA. Even though orally administered CII shows an active suppression of arthritis in the animal model of RA, the oral tolerance using CII in human RA is not consistent between the experiments. Barnett *et al* have demonstrated that oral CII therapy is effective in most RA patients (32). In contrast, Sieper *et al* have reported that only a minority of patients responded to treatment with oral CII (33). Thus, it is unclear whether oral tolerance induction using CII could be an effective strategy in the treatment of human RA.

25 Controversial effect of oral tolerance therapy in human disease is also noted in other autoimmune diseases. This may be attributed to the insufficient antigen delivery into Peyer's patches in the intestine, main machinery of oral tolerance induction. Unfortunately, only very few attempts to overcome the intraluminal

digestion and to enhance the rate and amount of antigen have been made in tolerance induction. Although the particles composed of polymer and protein have been proved for their usefulness for protein delivery, there has been no attempt to test or suggestion of the ability of PLGA particles or of active ingredients-entrapping PLGA particles to induce tolerance has been made.

Additional variable to determine the usefulness of tolerance therapy is easy availability of autoantigens including CII, MBP, S-antigens, and glutamic acid decarboxylase (GAD). Since these antigens should be purified from live animals to administer into human, they are expensive and have a risk of transferring some viruses contained in animals to human. In this respect, practical usefulness of these antigens is limited in treating human disease. Recently, it has been reported that the synthetic peptides encompassing immunodominant portions of afore-mentioned autoantigens, also can suppress the development of autoimmune disease, just like a whole molecule of antigen (34, 35, 36, 37 and 38).

Synthetic peptides may be utilized to treat autoimmune diseases such as RA, multiple sclerosis, and uveitis, without the drawbacks associated with the difficulty in purifying autoantigens from animals. Tolerance induction using synthetic immunodominant peptides seems to be promising since these peptides may be prepared in an easy and economic way as well as they have no risk to transfer viral diseases.

The inventors conducted a series of oral tolerance induction using biodegradable polymer particles with or without an autoimmune antigen or its immunodominant peptide fragments entrapped therein in an animal experimental model of RA, a representative autoimmune disease, and tested the ability of polymer particles to induce tolerance and mechanisms thereof by immunologic assays and electron microscopy, and as result thereof, found out that the polymer particles can induce oral tolerance against autoimmune diseases and enhance oral tolerance of autoimmune antigens.

SUMMARY OF THE INVENTION

The present invention provides a method for treating autoimmune diseases, which comprises administering orally to a mammal suffering from autoimmune diseases particles of biodegradable polymers, in particular poly(DL-lactide-co-glycolide) in an amount of effective to induce tolerance against autoimmune response.

The present invention further provides a method for treating autoimmune diseases, which comprises administering orally to a mammal suffering from autoimmune diseases particles of biodegradable polymers entrapping an autoimmune antigen, said particles being administered in an amount of effective to induce tolerance against autoimmune response.

The present invention still provides a use of biodegradable polymers as an inducer or enhancer for oral tolerance against autoimmune diseases.

The above and other objects, features and applications of the present invention will be apparent to those of ordinary skill by the following detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a SEM(Scanning Electron Microscopy) analysis depicting the PLGA particles entrapping type II collagen(PLGA-CII), in which the arrows indicate the diameter of a particle, approximating 300nm;

Figure 2 is *in vitro* release of CII from PLGA particles, showing a slow release profile over 4 weeks and typical biphasic pattern;

Figure 3(a) and 3(b) are graphs depicting the severity and incidence of arthritis with the lapse of time in a mice having a collagen-induced arthritis, respectively;

Figure 4 is a clinical assessment of arthritis in mice fed with PLGA-CII, native

The present invention provides a use of biodegradable polymer particles, in particular polylactide-polyglycolide (PLGA) particles as an inducer of oral immunological tolerance, especially for autoimmune diseases.

The biodegradable polymers may contain an autoimmune antigen entrapped
5 therein to enhance oral tolerance in mammals.

The term of 'autoimmune antigen' used herein means tissue-specific antigens that are themselves the subject of autoimmune attack and may include, among others, antigens of EAE, CIA, uveitis, multiple sclerosis, insulin dependent diabetes mellitus (IDDM) and the like. In specifically, it include type II collagen, major basic protein
10 (MBP), glutamic acid decarboxylase (GAD), S antigen and the like as well as immunodominant peptide fragments thereof. The immunodominant peptide fragments are known to those skilled in the art and may include the following SEQ. ID. NO. 1 through SEQ. ID. NO. 11 as well as their equivalents as long as they retain immunogenic property :

15 1) Rheumatoid arthritis (35)

CII (74-93); ARGFPGTPGLPGGVKGHRGYP (SEQ. ID. NO. 1)

CII (254-273); TGGKPGIAGFKGEQGPKGEP (SEQ. ID. NO. 2)

CII (924-943); PGERGLKGHRGFTGLQGLPG (SEQ. ID. NO. 3)

CII (255-270); CGEBGIAGFKGEQGPK (SEQ. ID. NO. 4)

20 2) Multiple sclerosis (36,37)

Myelin proteolipid protein (PLP) (139-151); HSLGKWLGHDPKF
(SEQ. ID. NO. 5)

PLP (178-191); NTWTTCQSIAFPSK (SEQ. ID. NO. 6)

PLP (258-273); IAATYNFAVLKLMGRG (SEQ. ID. NO. 7)

25 Major basic protein (87-99); VHFFKNIVTPRTP (SEQ. ID. NO. 8)

Myelin oligodendrocyte glycoprotein (92-106); DEGGYTCFFRDHSYQ
(SEQ. ID. NO. 9)

3) Autoimmune Uveitis (38)

S antigen (343-362); LGELTSSEVATEVPFRLMHP (SEQ. ID. NO. 10)

4) IDDM (42)

Glutamic acid decarboxylase-65 (524-543); SRLSKVAPVIKARMMEYGTT

5 (SEQ. ID. NO. 11)

The sequence of the immunodominant peptide fragments may be slightly varied depending on their origins, and the present invention encompasses the polymer particles entrapping the peptide fragments regardless of their origin as long as they show the tolerance inducing property in living body. Both of synthetic and naturally
10 occurring peptide fragments may be employed according to the present invention, and synthetic peptide fragments are advantageously used due to easy and unexpensive production thereof.

The term of 'autoimmune disease' used herein means a condition where the immune system mistakenly perceives the body's own tissue as foreign and mounts an
15 abnormal immune response against it and may include experimental allergic encephalomyelitis (EAE), collagen induced arthritis (CIA), rheumatoid arthritis (RA), uveitis, multiple sclerosis, IDDM, non-obese diabetes, and the like. Rheumatoid arthritis can be advantageously treated by the present invention. In particular, the polymer particles without antigen can advantageously be used to treat Th1-mediated
20 or T cell-mediated autoimmune diseases such as rheumatoid arthritis, IDDM, uveitis, multiple sclerosis, autoimmune thyroiditis (e.g., Grave's disease, Hashimoto's thyroiditis), autoimmune hepatitis, interstitial pneumonitis or glomerulonephritis, and their corresponding diseases in animal models.

Throughout the application, the term of 'polymers' or 'PLGA', or the part of
25 'PLGA' in the terms of 'PLGA-CII' (or 'PLGA entrapping CII'), or 'PLGA-CII(amino acid number)' should be understood to indicate particles made therefrom having the desired size unless they are intentionally used to stand for the compound

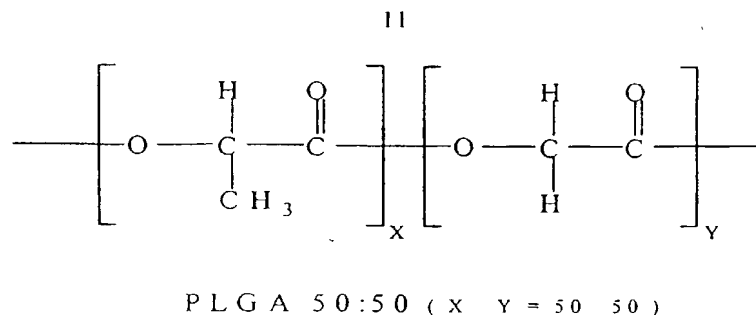
itself. Moreover, the term of 'particle(s)' is used to indicated to include both the polymer particles and the antigen-entrapping polymer particles, unless indicated otherwise.

The autoimmune antigen which will be entrapped by biodegradable polymers
5 may be selected by those skilled in the art without difficulty depending on the kind of the autoimmune diseases to be treated. For example, CII or its immunodominant peptides will be entrapped for treating RA in human and collagen-induced arthritis in animal models (rat, mouse, monkey, etc); major basic protein and its immunodominant peptide for multiple sclerosis in human and experimental
10 autoimmune encephalitis in animal models (rat, mouse, etc); GAD for IDDM in human and experimental diabetes mellitus in animal models (rat, mouse, etc); and S antigen for autoimmune uveitis in human and autoimmune uveitis in animal models (rat, mouse, etc).

The mammals to which the present invention can be applied may include, but
15 not limited to, rat, mouse, monkey, various experimental animal models, and human.

The biodegradable and biocompatible polymers which may be employed according to the present invention includes, but not limited to, poly(DL-lactide), polyglycolic acid, poly(DL-lactide-co-glycolide)('PLGA'), poly(epsilon-caprolactone) and the like. The PLGA is preferred for use in accordance with the
20 present invention since its safety in living body is well established. Polymeric materials for preparation of the particles are available commercially. These biodegradable polymers degrade through hydrolysis of polymer linkages to yield biocompatible products such as lactic acid and glycolic acid for PLGA, upon exposure to water.

25 The term of 'PLGA' is used herein to refer to the various copolymers of lactic and glycolic acids, which can be represented by following general formula:



The molecular weight of the polymers ranges from about 25,000 Daltons to about 120,000 Daltons. The ratio of lactide units to glycolide units ranges from 100:0 to 50:50. For example, PLGA having a ratio of lactide : glycolide = 85:15, which can be obtained from Birmingham Polymers, Incorporate, has an inherent viscosity of 0.58 dL/g and a molecular weight of approximately 90,900 daltons. It has been reported to have a biodegradation time up to approximately 10 months, depending on surface area, porosity and molecular weight. On the other hand, 50:50 PLGA polymer has a reported degradation time of 2 months.

The particles of biodegradable polymers (hereinafter, referred sometimes as simply 'particles') have a diameter ranging from one hundred nanometer to several thousand nanometers, preferably less than about 500nm, and more preferably about 200 to about 300nm. The particles may be prepared by conventional method, for example, by dispersing an organic solution of the polymer into an aqueous solution and then evaporating the solvent.

The particles entrapping an autoimmune antigen may be prepared by conventional water-in-oil-in water emulsion solvent evaporation methods. For example, the biologically active ingredients in a suitable aqueous solvent is emulsified with a solution of biodegradable polymers in an organic solvent such as methylene chloride to form an oil-in-water (o/w) emulsion. Then the o/w emulsion is emulsified with an aqueous solution of water-soluble polymer (e.g. polyvinyl alcohol) to give a w/o/w emulsion. Then the resulting w/o/w emulsion is evaporated

to remove the organic solvent. When immunodominant peptide fragments are used as an autoimmune antigen, they may advantageously be coupled in advance to a proper polymer such as PEG (MW more than 2,000) or polypeptides in order to moderate their release rate from the particles. PEG may be advantageously used for
5 this purpose.

The particles have preferably a size of less than 500nm, and more preferably between 200nm and 300nm. The loading of antigen varies between about 0.5% and about 10%. Particles with desired size were obtained by optimizing various formulation parameters such as volume and concentration of each solution used, a
10 kind of surfactant materials, and time period of energy input, energy sources such as sonicator, homogenizer or vortex used to form the water/oil/water emulsions.

The particles without or with an antigen entrapped therein may be administered orally to mammals such as human, cats, dogs, rats, mice, monkeys and the like for the purpose of treating autoimmune diseases.

15 The dose of the particles for oral administration may vary and be determined by those skilled in the art depending on the kind and severity of the diseases and condition of the subjects. For example, to treat RA, the dose may be within the range 0.1 - 1000mg of PLGA/kg of weight, and preferably 1 - 100 mg of PLGA/kg of weight, and more preferably 10 - 40mg of PLGA/kg of weight.

20 When the particle entrapping CII or its immunogenic peptide fragments is used, the dose of antigen may be within the range of 1 - 10000 μ g, preferably 10 - 1000 μ g, and more preferably 20 - 160 μ g.

The particles without or with an entrapped antigen may be administered orally and only single administration thereof can give a significant and satisfactory
25 suppression of autoimmune response in mammals.

Examples

The present invention will be described in more detail by way of various

Examples, which should not be construed to limit the scope of the present invention.

MATERIALS AND METHODS

Animal

DBA1 mice obtained from Jackson Laboratories (Bar Harbor, ME) were
5 maintained in groups of three to five in polycarbonate cage and fed standard mouse
chow and water adlibitum. The environment was specific pathogen-free for the mice
obtained from Jackson Laboratories. Neonatal mice were obtained by breeding mice
in inventor's facility. Mice were immunized at 8-12 weeks as described previously
(25, 27, 28).

10 **Preparation of type II collagen**

Intact bovine CII was extracted and purified from articular cartilage of calf as
described previously (39). Following steps were carried out; solubilization with
pepsin from cartilage slices after removal of proteoglycans by exposure to neutral 4
M guanidine HCl, repeated precipitation from 0.5 M acetic acid at 0.86 M NaCl, and
15 dialysis against 0.02 M Na_2HPO_4 ; removal of pepsin and proteoglycans by DEAE
chromatography; and removal of type I collagen by differential salt precipitation at
neutral pH. Purity was confirmed by SDS-PAGE. Lyophilized native bovine CII was
dissolved in 0.1N acetic acid at 1mg/ml, dialyzed against 50 mM Tris, 0.2M NaCl,
and then sterilized by filtering through a 0.2 μm micropore filter.

20 **Preparation of PLGA entrapping CII**

Entrapping of CII within PLGA was performed as described previously (40).

A solution of bovine CII (10 % w/v) in 0.5 M acetic acid (internal aqueous phase) was initially emulsified with a solution of PLGA (3 % w/v) in methylene chloride (oil phase), using a probe sonicator to form a water-in-oil (w/o) emulsion. The resulting emulsion was added to a 5% (w/v) PVA solution (external water phase) followed by
 5 second emulsification, resulting in a water-in-oil-in water (w/o/w) emulsion. The w/o/w emulsion was stirred over a magnetic stir plate overnight at room temperature to evaporate methylene chloride. Upon evaporation the volatile organic solvent was removed, resulting in an aqueous suspension of particles. Particles with proper size were then recovered by ultracentrifugation. The particles were washed twice with
 10 water and resuspended in water prior to lyophilization. The particles are sometimes referred to as 'PLGA-CII' for simplicity. Figure 1 is an electroscopic picture of the PLGA particles having a size of about 300nm.

Peptide synthesis and entrapment of peptide with PLGA.

Immunodominant peptide fragment of bovine CII (255-270) :
 15 CGEBGIAGFKGEQGPK (SEQ. ID. NO. 4) was synthesized and then entrapped with PLGA by organic solvent evaporation methods (40). Synthetic peptide was conjugated with polyethylene glycol (PEG, MW5000), because this peptide was short and not soluble in methylene chloride which was an organic solvent used in making particle. Conjugation of peptide with PEG (methoxy-PEG-NH₂) was as follows. The
 20 linker of SPDP (N-Succinimidyl 3-[2-pyridyldithio] propionate) was used in conjugation of peptide with PEG. 30mg of PEG was dissolved in 0.2ml of phosphate buffer (20mM, pH 7.5) and 5mg of SPDP in 0.2ml of DMSO. The two solutions were mixed and incubated at room temperature for 1 hour. After the reaction, SPDP-conjugated PEG was separated by gel filtration column chromatography eluted with
 25 distilled water at the flow rate of 0.5 ml/min, followed by freeze-drying the separated fractions. The resulted PEG-SPDP was dissolved with peptide (10mg) in 0.5ml of

phosphate buffer (20mM, pH 7.5), and incubated at room temperature overnight. The PEG-conjugated peptide was applied to the same gel filtration chromatography eluted with distilled water at the same flow rate (0.5ml/min). The purified fractions were freeze-dried and stored at -20°C.

5 PLGA entrapping peptide was prepared as the same method as described for the preparation of PLGA-CII. The solution of PEG-peptide(2.5mg) and PLGA (50mg) in methylene chloride (1.5ml) was emulsified with a solution of 5% PVA, using a probe sonicator for 20 seconds. The emulsion was stirred over a magnetic stir plate for 5 hours at room temperature to evaporate the organic solvent. After the
10 evaporation, the suspension was centrifuged at $500 \times g$ for 5 min to remove the particles. The resulting particles were washed twice by ultracentrifuging and resuspending, and lyophilized. The particles are sometimes referred to as 'PLGA-CII(255-270)' for the simplicity.

15 Induction of CIA and clinical evaluation of arthritis

Mice were immunized with native CII at 8 to 12 weeks of age. CII dissolved in 0.1 N acetic acid (2mg/ml) was emulsified (1:1 ratio) with CFA (Complete Freund Adjuvant) at 4°C. Mice were received 0.1ml of the emulsion containing 100 μ g CII into base of tail as a primary immunization. Booster injection(50 μ g CII) was given
20 into footpad on day 14. Mice were observed two to three times per week for onset, duration, and severity of joint inflammation over a period of 12 weeks after primary immunization. Each limb was assessed on a 0- to 4-point scale by the following criteria: 0 = normal; 1 = mild inflammation of a single area (i.e., either the midfoot, ankle, or toes); 2 = moderately severe arthritis involving toes and ankle or midfoot;
25 3 = severe arthritis involving entire paw; and 4 = severe arthritis resulting in ankylosis and loss of joint movement. Since the left foot that was used for booster immunization was excluded from the evaluation, the possible maximum arthritis

score was 12. Mean arthritic index, incidence of arthritis (%), and percent arthritic limbs were used for comparison and calculated as follows:

Mean arthritis index = Sum of the arthritic score / Total number of mice

Incidence of arthritis (%) =

$$5 \quad \frac{\text{Number of mice with arthritic score} > 2 \text{ in any limb}}{\text{Total number of mice}} \times 100$$

Percent arthritic limbs (%) =

$$10 \quad \frac{\text{Number of limbs with arthritic score} > 2}{\text{Total number of limbs}} \times 100$$

Tolerance induction

Mice were fed 8 times with 5 μ g CII on days -17, -15, -13, -11, -9, -7, -5, -3 relative to the primary immunization with CII in CFA on day 0. Control mice were fed 8 times with 0.1 N acetic acid. PLGA, PLGA-CII 40 μ g (cumulative dose of CII in mice repetitively fed with CII), or PLGA-CII(255-270) 4 μ g were fed only once on day -17.

Assessment of T cell proliferation

Draining or mesenteric lymph node, and spleen were removed from animal at 14 days after immunization and dissociated and washed in RPMI 1640. Lymph nodes and spleen were individually minced into single cell suspensions in RPMI 1640 and washed three times. Cells (5 X 10⁵/well) were cultured in 96-well microtiter plates

(Nunc, Roskilde, Denmark) with or without 100 μ g/ml of CII in 0.3 ml of Click's medium supplemented with 0.5% mouse serum at 37°C, 5% humidified CO₂ for 4 days. Eighteen hours before the termination of the cultures, 1 μ Ci [³H] thymidine was added to each well. Cells were harvested onto glass fiber filters and counted on a
5 Matrix 96 direct ionization β -counter (Packard Instrument Co, Meridan, CT). The results are presented as stimulation indices (SI), calculated as the ratio of cpm in the presence of antigens / cpm without antigens.

Assay for IgG antibodies to bovine native type II collagen

Sera were collected on day 28 after primary immunization and stored at -20°C
10 until the assay. The levels of IgG anti-CII in sera were determined by enzyme-linked immunosorbent assays kit (Chondrex, Redmond, WA). The optical density of standard serum which was serially diluted 2-fold was expressed as 100, 50, 25, 12.5, 6.25, 3.125 arbitrary units (a.u.), respectively, according to the manufacture's instructions. The relationship of the optical density measured in standard serum
15 diluted serially and arbitrary unit showed a good linear correlation in all determinations ($r>0.98$, data not shown). The concentrations of IgG anti-CII in the sera diluted 1: 4000 are presented as relative values (a.u.) compared to the optical density of the standard serum which was diluted serially.

Immunohistochemical staining of CII in Peyer's patch

20 To determine whether CII entrapped within PLGA is still present in the intestine for a long period, immunohistochemical staining of CII was performed in Peyer's patches. Briefly, Peyer's patches were obtained from mice administered orally PLGA entrapping CII on days 14 and 35 after feeding. Peyer's patches from mice fed with repetitive CII or acetic acid was used for control tissue. After fixing with

periodate-lysine paraformaldehyde solution at 4°C overnight, tissues were dehydrated with alcohol. After washing, wax (polyethylene glycol 400 distearate) embedding and microsection (5 μ m) of tissue were done. To remove peroxidase activity within tissue, methanolic H₂O₂ was added and then tissues were blocked with normal sera for 1 hour. After tissue were incubated overnight with goat monoclonal antibodies to bovine CII, generous gifts from Dr. Andrew H Kang, DAG (peroxidase conjugated F(ab')₂, Donkey anti-goat IgG, Jackson Immunoresearch Laboratories) was added to tissue. Biotinylated antibody was then added to tissues and color reaction was detected with diaminobenzidine (DAB) on microscopy.

10 ***In vitro* release of entrapped antigen from PLGA**

The *in vitro* release of CII from PLGA particles was evaluated for 4 weeks under physiologic conditions at 37°C. FITC-labeled collagen was used in *in vitro* release test to increase sensitivity. After labeling collagen with FITC, the particles were prepared as described above. Then, the particles were dispersed in phosphate buffer (PH 7.4, 0.1M) and maintained at 37°C with mild stirring. Keeping the sample in this condition, an aliquot of the sample was taken and its fluorescence was measured by spectrofluorometer periodically.

Characterization of particles by electron microscopy

Scanning electron microscopy (SEM) was performed to determine the surface topography of the particles. Particles were gold coated using a sputter gold coater (Denton Vacuum Inc, Cherry Hill, NJ) at 40 miliampere current and 50 millitorr pressure for 200 seconds to the thickness of 300 Å. Samples of gold coated particles were cooled over dry ice prior to SEM observations to avoid their melting under magnification due to electron beam exposure (Hitachi S570, Chery Hill, NJ). To

characterize PLGA particles *in vivo* and to determine whether PLGA particles are still present in the intestine for a long period, a ultra-thin frozen section of Peyer's patches was obtained on days 14 and 35 after feeding of PLGA-CII and then the presence of PLGA particles was investigated on electron microscopy.

5 Statistics

Comparisons of numerical data between groups were performed by the student T-test and ANOVA and for categorical data by a chi-square test or Fisher's exact probability test. P values less than 0.05 were considered significant.

RESULTS

10 1. Characterization of PLGA particles

Particles with desired size were obtained by optimizing various formulation parameters and energy source input used to form the water/oil/water emulsions. As can seen from the SEM analysis, nanoparticles were spherical with smooth uniform surfaces with more than 80% particles in the desired size range. Discrete spherical
15 particles had a mean diameter of 299.7 ± 4.9 nm (Figure 1). To find out collagen loading of particles, weighed particles were dissolved in 0.4 N NaOH, 10 % SDS (w/v) solution. And then, the solution was assayed for collagen by Lowry method. 1.2 % of total weight was composed of collagen. Encapsulation efficiency was approximately 6.5%.

20 This study demonstrated that PLGA particles produced *in vitro* an initial burst release of 10 to 20% of encapsulated CII within two days. The subsequent release of CII up to 20 days was at a slower releasing rate during which 10% to 20% of CII was

released. The slow release phase was followed by a second burst release from day 15 to 30 possibly due to hydrophobic erosion of the polymer matrix (Figure 2).

2. Establishment of collagen induced arthritis

Figure 3(a) and 3(b) shows the incidence and severity of collagen-induced arthritis with the lapse of time in an animal model, respectively.

As shown in Figure 4(b) and 4(c), in mice (n=9) fed acetic acid and injected with CII and CFA, CIA developed from 3 weeks. The severity and incidence of arthritis peaked at 5 weeks, reaching 5.1 of mean arthritic index (maximum 12) and 100% of incidence, respectively and were decreased over time thereafter, which is consistent with previous reports (25, 27, 28).

3. Tolerance induction with PLGA entrapping CII

To test the ability of single administration of PLGA-CII to suppress CIA, the mean arthritis index, incidence, and number of arthritic limbs were compared between mice (n=8) fed with PLGA-CII once and those fed with eight times of CII (n=8) or acetic acid (n=8), at five weeks after primary immunization. As shown in Figure 5, mice fed with PLGA-CII and mice fed with CII had significant lower levels of the mean arthritis index (PLGA-CII 0.9 ± 0.3 , CII 2.8 ± 0.9 , acetic acid 4.5 ± 1.1 , $p < 0.001$), incidence (PLGA-CII 0%, CII 50%, acetic acid 100%, $p < 0.001$), and number of arthritic limbs (PLGA-CII 0/24 [0%], CII 5/24 [20.8%], acetic acid 14/24 [58.3%], $p < 0.001$) compared to those fed with acetic acid. Comparison of clinical parameters between PLGA-CII and CII fed mice showed that mice fed with PLGA-CII had significant lower levels of the mean arthritis index ($p < 0.001$), incidence ($p = 0.02$), and number of arthritic limbs ($p = 0.008$) compared to those fed with CII. These observation demonstrated that the single administration of PLGA-CII

5 4. Tolerance induction using PLGA entrapping CII (255-274).

5. Assessment of the ability of PLGA to induce tolerance

In addition to the slow release property of entrapped protein, several kinds of particulate antigen are known to have a potential to modulate immunogenicity by itself. To assess the ability of PLGA itself to induce tolerance, the inventors conducted a series of CIA induction in mice fed with PLGA. As a result, mice fed once with PLGA (n=8) had significant lower levels of mean arthritis index and percent arthritic limbs compared to mice fed with acetic acid (n=7) when assessed at

5 weeks after primary immunization (mean arthritic index; 1.8 ± 0.8 versus 3.9 ± 1.9 , $p=0.019$, percent arthritis limb; 10/21 [12.9%] versus 3/24 [42.9%], $p=0.01$) (Figure 7(a)).

The incidence of arthritis also tended to be lower in mice fed with PLGA than those fed with acetic acid ($p=NS$). Comparison of clinical parameters at 5 weeks between PLGA and PLGA-CII administered mice showed that the incidence of arthritis was significantly lower in mice fed with PLGA-CII than PLGA alone (0% versus 50%, $p=0.03$). Mean arthritis index and percent arthritic limb tended to be lower in PLGA-CII fed mice, but they did not reach statistical significance (Figure 7(b)). These data demonstrated that PLGA alone could suppress CIA, but its potency was more enhanced by CII entrapping. These observations are strong evidences that tolerogenicity by PLGA-CII is achieved by the mechanism of immunosuppressive effect of PLGA itself (tolerance inducing effect) as well as repetitive stimulation of intestinal immune cells by PLGA-CII as a carrier system (tolerance enhancing effect).

15 6. Effect of PLGA-CII on autoimmune response to CII

Circulating IgG antibodies to CII were significantly lower in CII-fed ($n=9$) and PLGA-CII-fed mice ($n=9$) compared to acetic acid-fed mice ($n=9$) (PLGA-CII; 6.4 ± 7.7 , CII; 15.2 ± 18.9 , acetic acid; 1.8 ± 0.8 , $p<0.01$). PLGA-fed mice tended to have lower levels of IgG antibodies to CII compared to CII-fed mice ($p=NS$). Assay for T cell proliferative responses to CII in draining or mesenteric lymph node and spleen also showed that mice fed with PLGA-CII, PLGA and CII had lower levels of T cell proliferative responses to CII (Figure 8(a) and 8(b)). These observations clearly showed that PLGA-CII or PLGA itself could suppress both humoral and cellular autoimmune responses systemically when administered orally, which constructs an important mechanism of PLGA to induce tolerance since both T cells responding to CII and IgG antibodies to CII, are known to play a critical role in

arthritis induction of susceptible animals.

7. Identification of PLGA particles in Peyer's patches

To characterize PLGA particles *in vivo* and to determine whether PLGA
 5 particles are still present in the intestine for a long period, the presence of PLGA
 particles was investigated in Peyer's patches on days 14 and 35 after feeding of
 PLGA, which is the time that any soluble antigen can not be detected in the intestine;
 by electron microscopy. As shown in Figure 9(b), PLGA particles having a size of
 about 300 nm were found in dendritic cells of Peyer's patches in an aggregated
 10 manner. They were located on the cytoplasm in an intact form as well as on
 lysozyme granules in a degraded form. Surprisingly, dendritic cells phagocytosing
 PLGA particles revealed an increased amount of lysozyme granules, indicating
 cellular activation.

However, these findings were not detected in Peyer's patches from mice fed
 15 CII or acetic acid, repetitively (Figure 9(a)). Recently, it is reported that hyperplasia
 or activation of dendritic cells plays a pivotal role in tolerance induction, in contrast
 with the generally accepted view of dendritic cells as immunostimulatory antigen
 presenting cells. Our data clearly showed that, like results from *in vitro* release test,
 PLGA particles were present in dendritic cells of Peyer's patches with a slow
 20 degradation, at least up to 35 days and could activate dendritic cells, which may
 explain how PLGA particles can suppress the systemic autoimmune response to CII
 as well as the incidence and severity of arthritis. This may be one of the possible
 mechanism that PLGA particles contribute to tolerance induction (tolerance induction
 effect).

25 8. Persistence of CII in Peyer's patches from mice single fed PLGA-CII

On CII specific immunohistochemical staining of Peyer's patches, we found that CII was densely stained around dendritic cells from PLGA-CII fed mice on days 14 and 35, which is the time that any soluble antigen can not be normally detected in the intestine, which is consistent with the findings in *in vitro* release test (Result 1, 5 Figure 3). However, these findings were not noted in mice fed with CII or acetic acid, repetitively (Figure 10). These findings demonstrated that CII was protected from acids and enzymatically hostile environments and released slowly from PLGA. This is also persuasive evidence that PLGA-CII contributes to tolerance induction in a manner of chronic antigenic stimulation of intestinal immune cells, driven by 10 long-lasting CII.

Although preferred embodiments of the present invention have been described in detail herein above, it should be clearly understood that many variations and/or modifications of the basic inventive concepts herein taught which may appear to those skilled in the art will still fall within the spirit and scope of the present 15 invention as defined in the appended claims.

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CLAIMS

1. (Amended) A method for treating autoimmune diseases, which comprises administering orally to a mammal suffering from autoimmune diseases particles of biodegradable polymers capable of reducing autoimmune response in an amount of
5 effective to induce tolerance against autoimmune response.
2. The method according to Claim 1, wherein said biodegradable polymers are poly(DL-lactide-co-glycolide), polylactides or polyglycolides.
3. The method according to Claim 1, wherein said autoimmune diseases are one of Th1-mediated or T cell-mediated autoimmune diseases selected from the
10 group consisting of rheumatoid arthritis, insulin dependnent diabetes mellitus, uveitis, multiple sclerosis, autoimmune thyroiditis, autoimmune hepatitis, interstitial pneumonitis and glomerulonephritis, and their corresponding diseases in animal models.
4. The method according to Claim 3, wherein said autoimmune disease is
15 rheumatoid arthritis.
5. The method according to any one of Claim 1 to Claim 4, wherein said mammal is human, rats, mice and monkeys.
6. The method according to any one of Claim 1 to Claim 4, wherein a single dose of said particles is administered to induce tolerance against autoimmune
20 response.
7. The method according to any one of Claim 1 to Claim 4, wherein said particles have a size of less than about 500nm.

- 6 - 31

ART 34 AMDP

10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100

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15. The method according to any one of Claim 8 to Claim 12, wherein said particles have a size of less than about 500nm.

16. (Amended) A composition for inducing tolerance for autoimmune diseases, which comprises as an active ingredient particles of biodegradable polymers
5 capable of reducing autoimmune response. in an amount of effective to induce tolerance against autoimmune response.

17.(Amended) A composition for inducing tolerance for autoimmune diseases, which comprises as an active ingredient particles of biodegradable polymers entrapping an autoimmune antigen in an amount of effective to induce tolerance
10 against autoimmune response, and said particles of biodegradable polymers capable of reducing autoimmune response.

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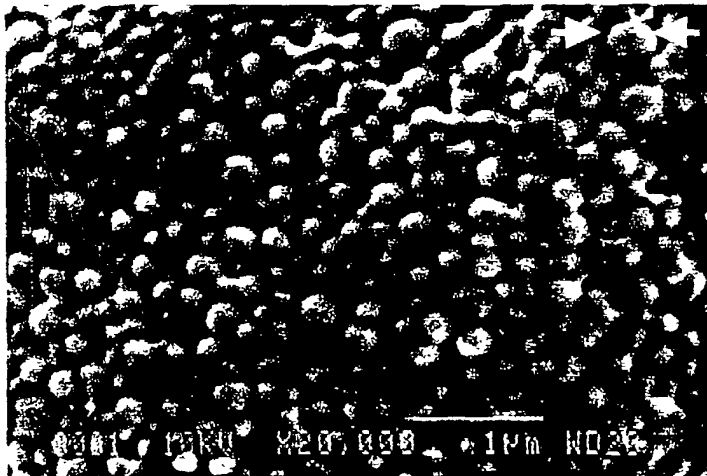
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(57) Abstract: A method for treating
autoimmune diseases by administering
orally to a mammal suffering from
autoimmune diseases particles of
biodegradable polymers or their complex
with an autoimmune antigen is provided.
Only single administration can effectively
induce oral tolerance to autoimmune
diseases, resulting in a strong and
prolonged suppress of the diseases.

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PCT/KR99/00460

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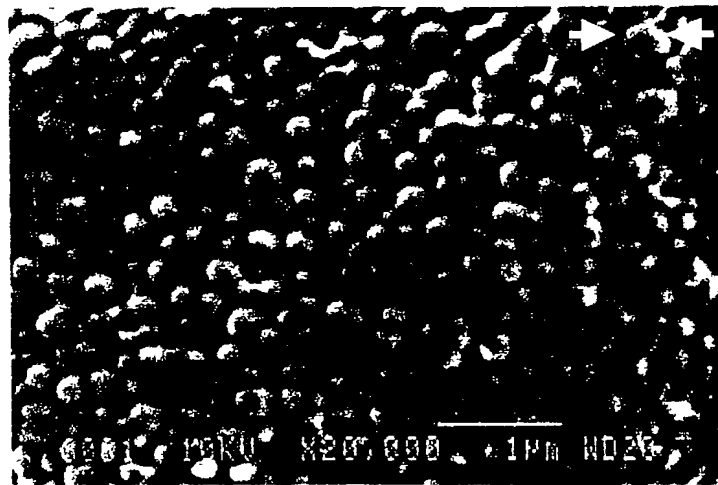
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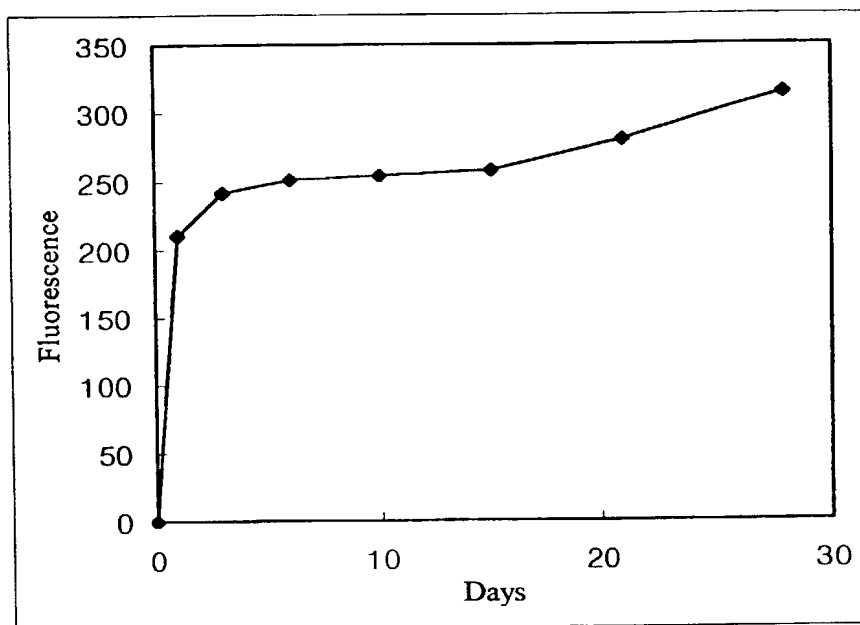
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FIG. 1



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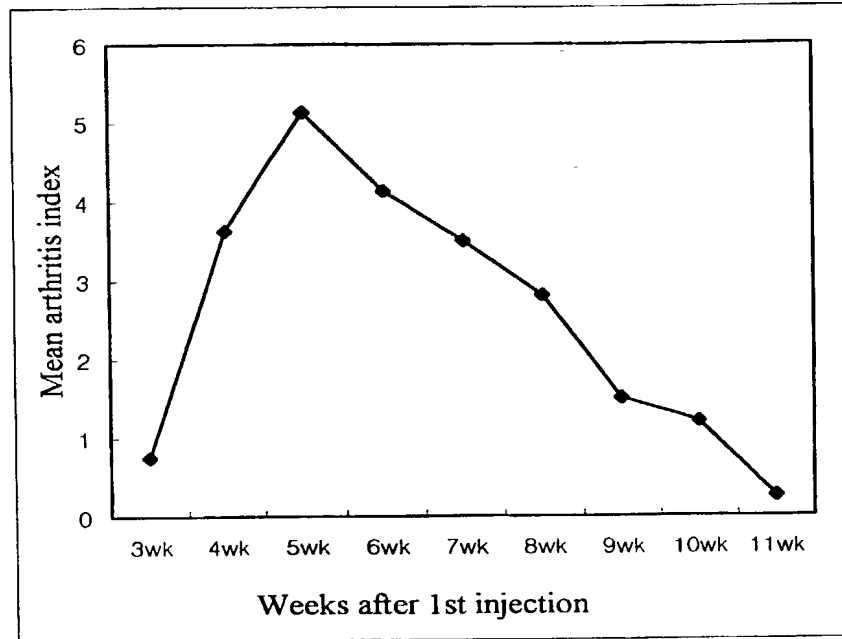
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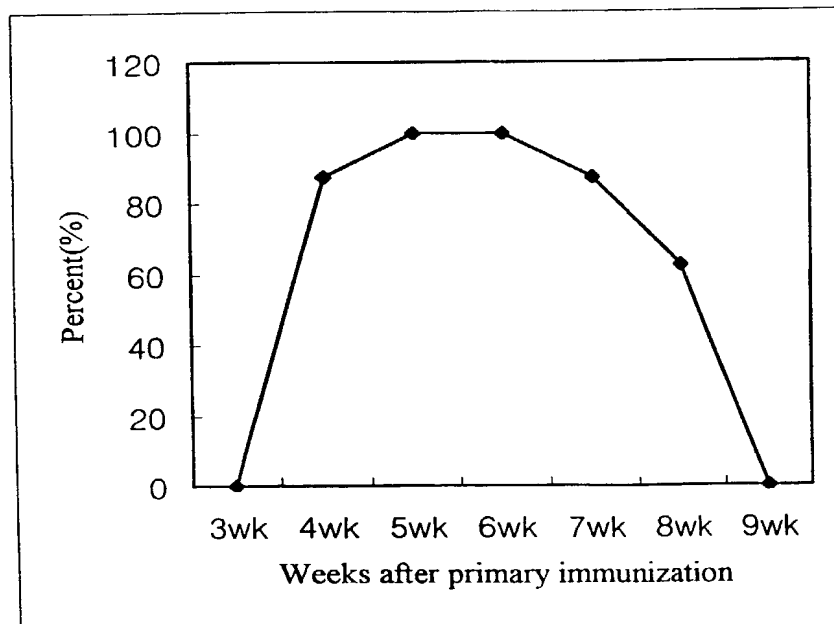
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FIG. 3

(a)



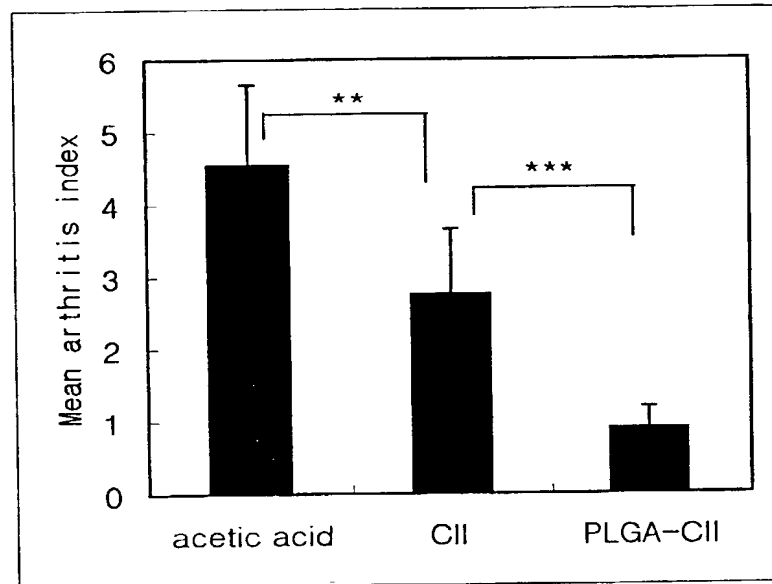
(b)



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FIG. 4

(a)



(b)

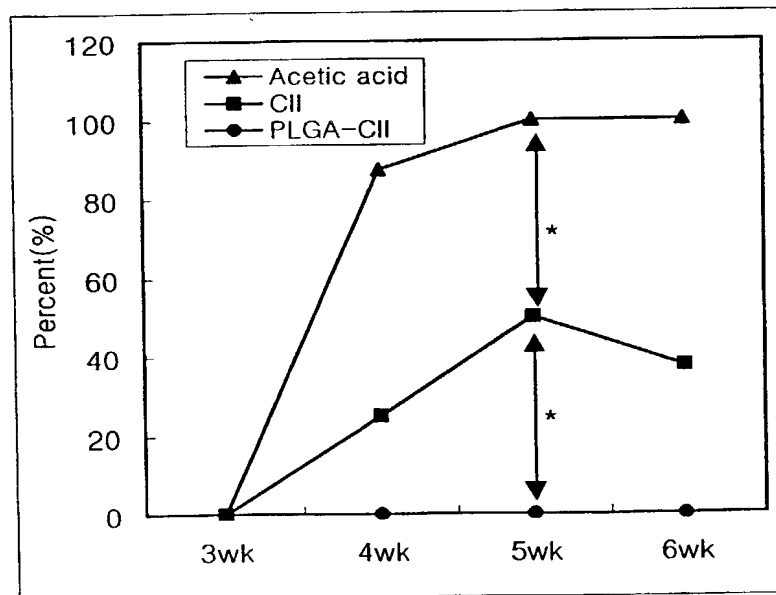
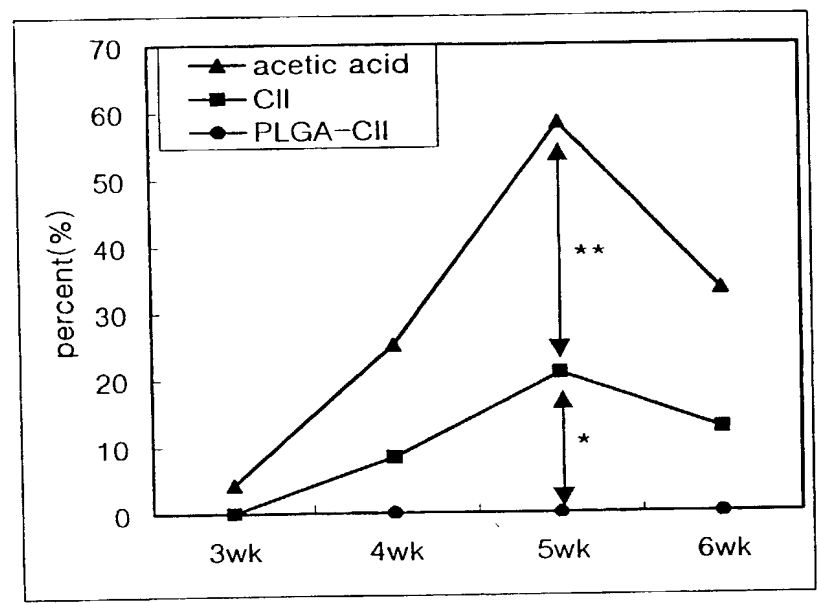


FIG. 4

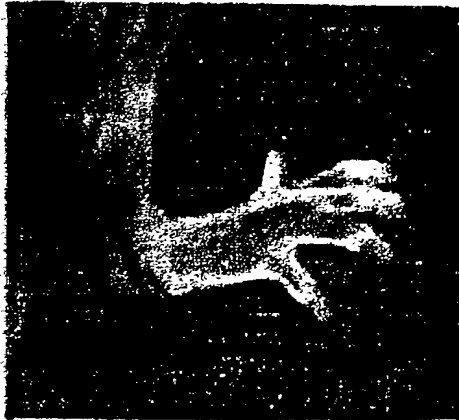
(c)



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FIG. 5

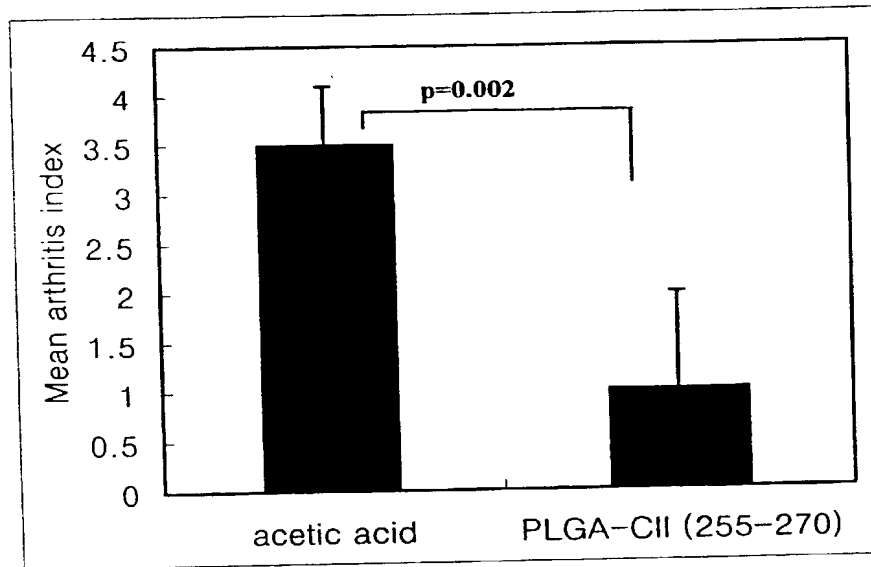
**Collagen Induced Arthritis in DBA/1 mice
(5wk after 1st injection)**

tolerized**non-tolerized**

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FIG. 6

(a)



(b)

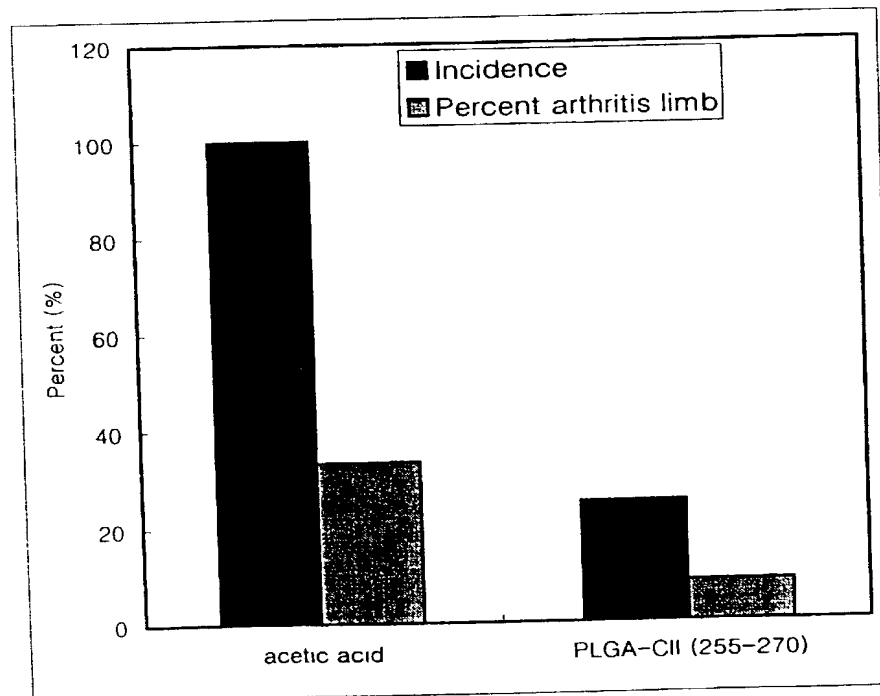
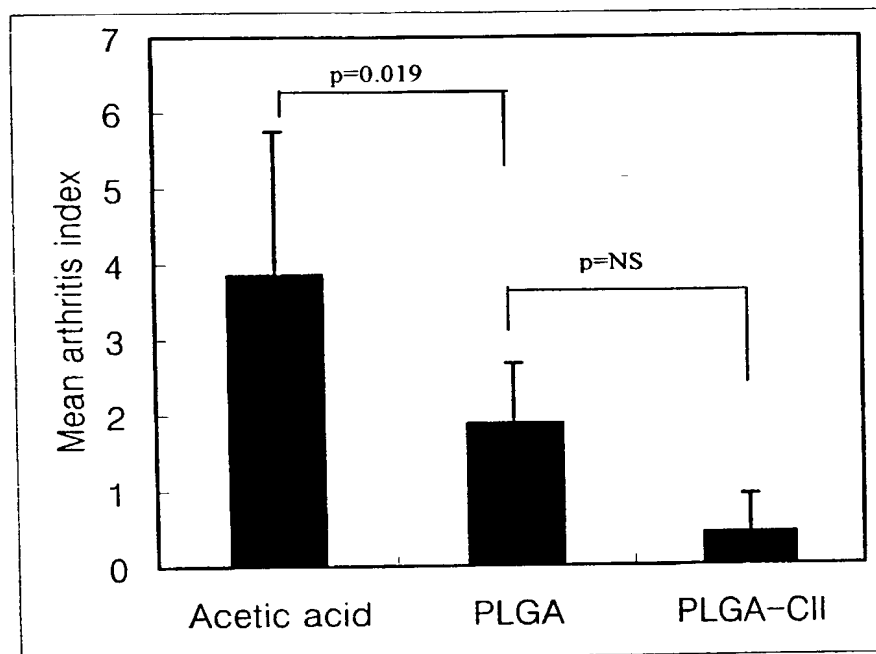


FIG. 7

(a)



(b)

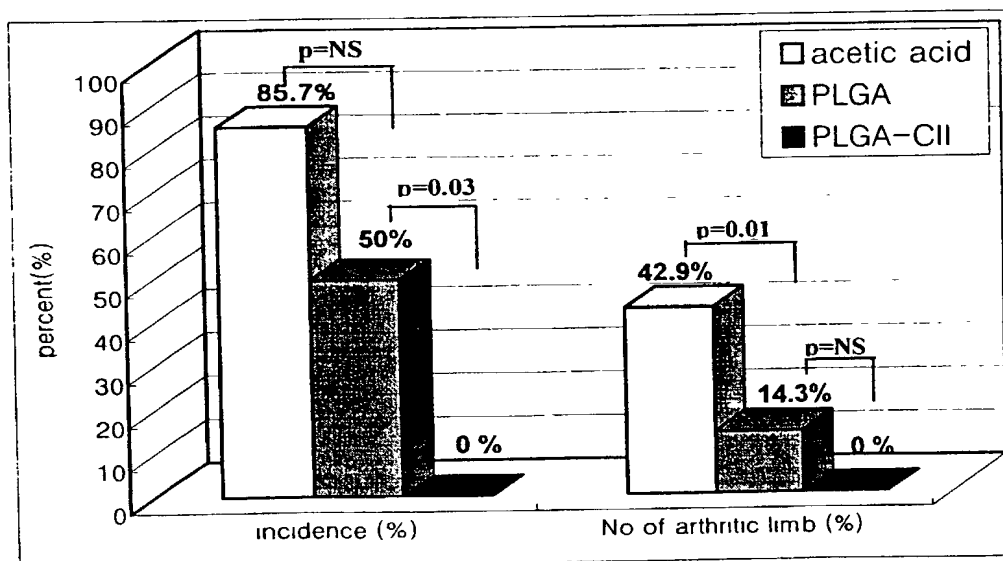
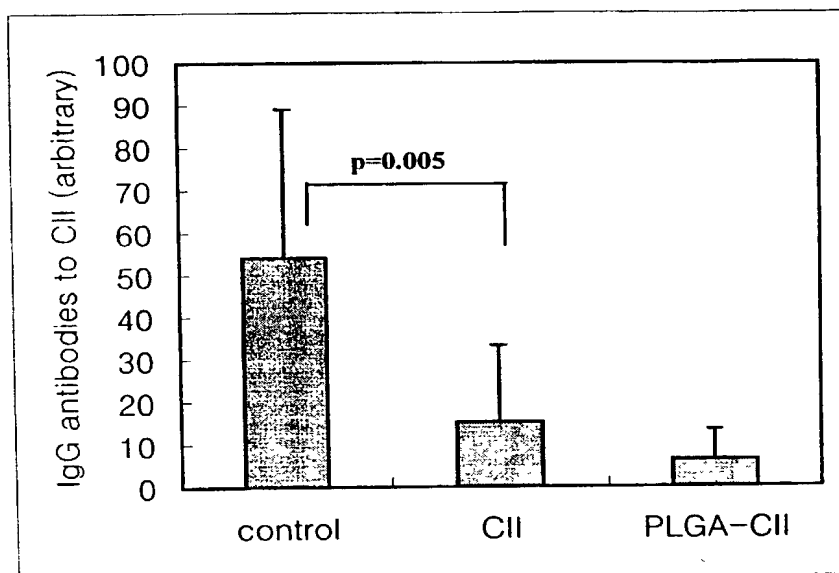
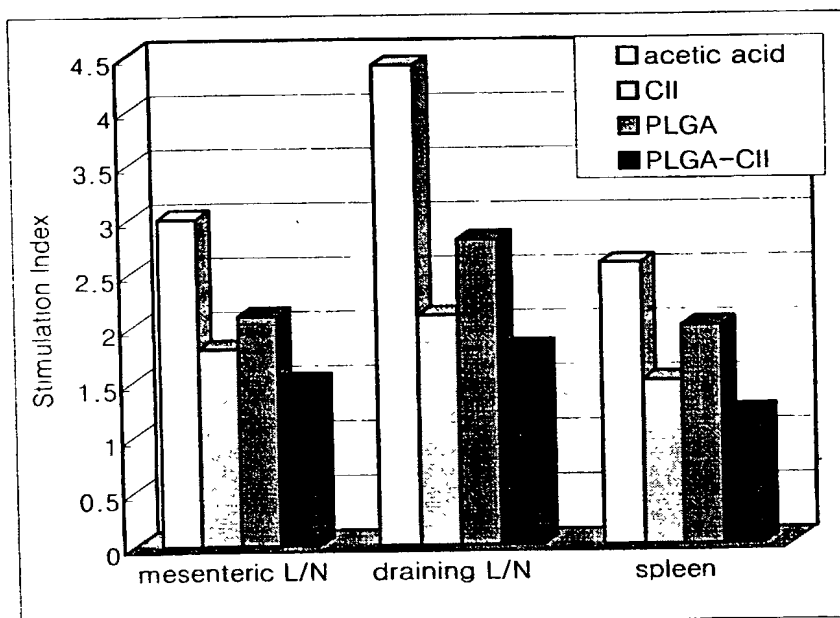


FIG. 8

(a)



(b)



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FIG. 9

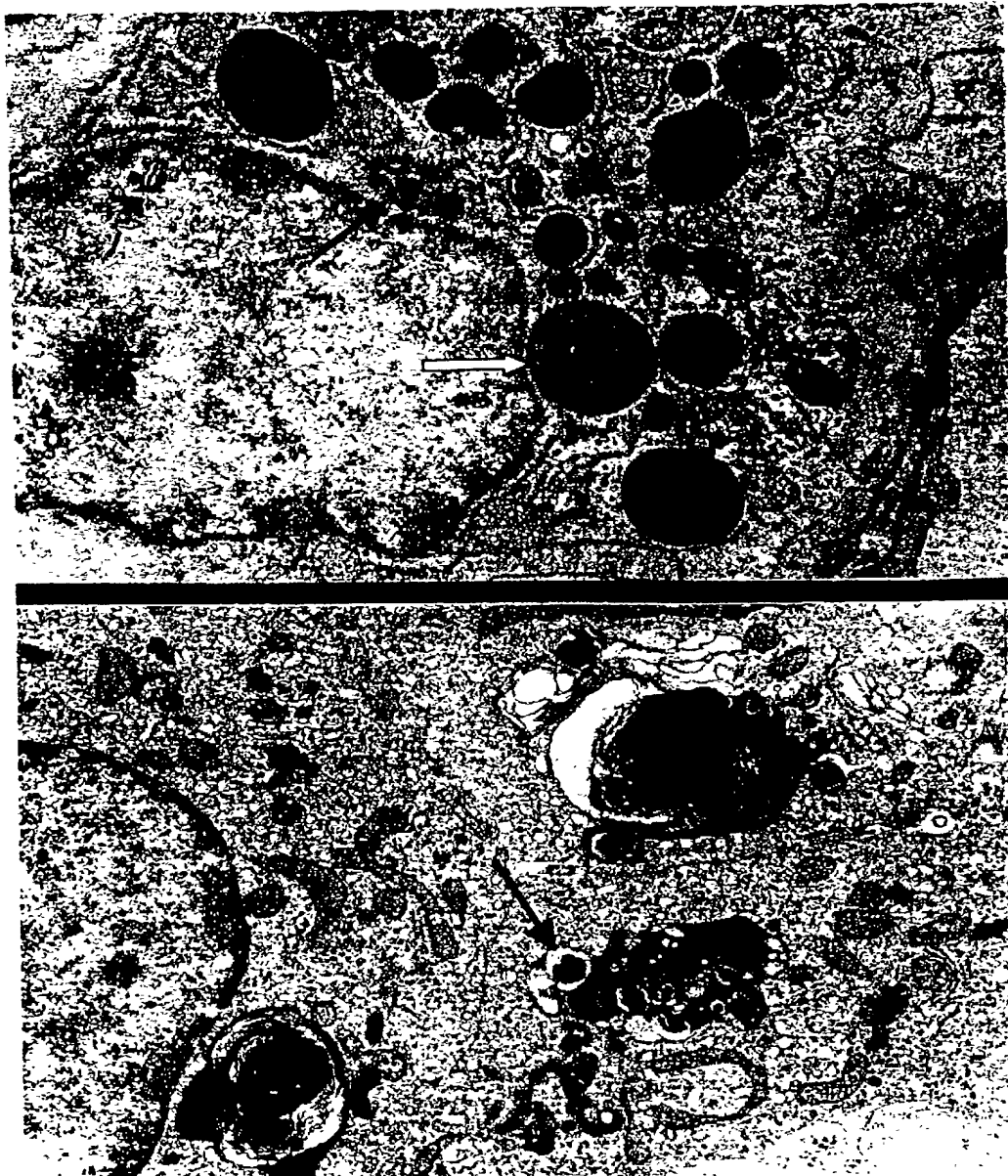
(a)



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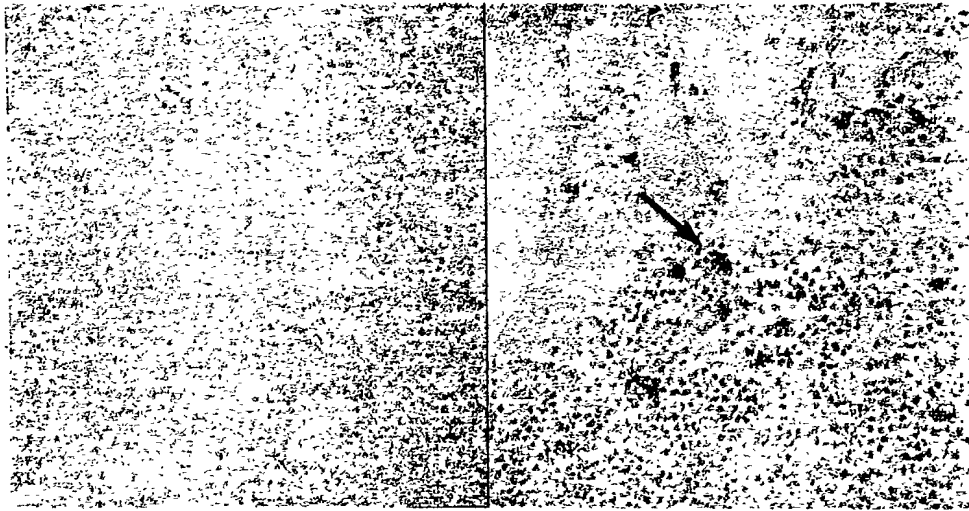
FIG. 9

(b)



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FIG. 10



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Telephone: (703) 205-8000 Facsimile (703) 205-8050

As a below named inventor, I hereby declare that: my residence, post office address and citizenship are as stated next to my name, that I verily believe that I am the original, first and sole inventor (if only one inventor is named below) or an original, first and joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled.

IMMUNOLOGICAL TOLERANCE-INDUCTION AGENT

the specification of which is attached hereto. If not attached hereto,

the specification was filed on _____ as
United States Application Number _____,
and amended on _____ (if applicable) and/or
the specification was filed on August 18, 1999 as PCT
International Application Number PCT/KR99/00460, and was
amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 56.

I do not know and do not believe the same was ever known or used in the United States of America before my or our invention thereof, or patented or described in any printed publication in any country before my or our invention thereof or more than one year prior to this application, that the same was not in public use or on sale in the United States of America more than one year prior to this application, that the invention has not been patented or made the subject of an inventor's certificate issued before the date of this application in any country foreign to the United States of America on an application filed by me or my legal representative or assigns more than twelve months (six months for designs) prior to this application, and that no application for patent or inventor's certificate on this invention has been filed in any country foreign to the United States of America prior to this application by me or my legal representatives or assigns, except as follows.

I hereby claim foreign priority benefits under Title 35, United States Code, § 19(a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)

Priority Claimed

(Number)	(Country)	(Month/Day/Year Filed)	<input type="checkbox"/>	<input type="checkbox"/>
			Yes	No
(Number)	(Country)	(Month/Day/Year Filed)	<input type="checkbox"/>	<input type="checkbox"/>
			Yes	No
(Number)	(Country)	(Month/Day/Year Filed)	<input type="checkbox"/>	<input type="checkbox"/>
			Yes	No
(Number)	(Country)	(Month/Day/Year Filed)	<input type="checkbox"/>	<input type="checkbox"/>
			Yes	No

I hereby claim the benefit under Title 35, United States Code, § 19(e) of any United States provisional application(s) listed below.

(Application Number)	(Filing Date)
(Application Number)	(Filing Date)

All Foreign Applications, if any, for any Patent or Inventor's Certificate Filed More than 12 Months (6 Months for Designs) Prior to the Filing Date of This Application:

Country	Application Number	Date of Filing (Month/Day/Year)
_____	_____	_____

I hereby claim the benefit under Title 35, United States Code, § 20 of any United States and/or PCT application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States and/or PCT application in the manner provided by the first paragraph of Title 35, United States Code, § 12, I acknowledge the duty to disclose information which is material to the patentability as defined in Title 37, Code of Federal Regulations, § .56 which became available between the filing date of the prior application and the national or PCT international filing date of this application

(Application Number)	(Filing Date)	(Status - patented, pending, abandoned)
(Application Number)	(Filing Date)	(Status - patented, pending, abandoned)

Attorney Docket No. 1599-0213P

I hereby appoint the practitioners at **CUSTOMER NO. 2292** as my attorneys or agents to prosecute this application and/or an international application based on this application and to transact all business in the United States Patent and Trademark Office connected therewith and in connection with the resulting patent based on instructions received from the entity who first sent the application papers to the practitioners, unless the inventor(s) or assignee provides said practitioners with a written notice to the contrary.

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P.O. Box 747 □ Falls Church, Virginia 22040-0747

Telephone: (703) 205-8000 □ Facsimile: (703) 205-8050

PLEASE NOTE:

YOU MUST
COMPLETE
THE
FOLLOWING:

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full Name of First
or Sole Inventor:
Insert Name of
Inventor
Insert Date This
Document is Signed

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Inventor, if any:
see above

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Full Name of Fourth
Inventor, if any:
see above

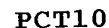
GIVEN NAME/FAMILY NAME <u>Euiyoung BAE</u>	INVENTOR'S SIGNATURE <u>[Signature]</u>	DATE* <u>Feb. 6. 2002</u>
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GIVEN NAME/FAMILY NAME <u>Woo-Kyoung LEE</u>	INVENTOR'S SIGNATURE <u>[Signature]</u>	DATE* <u>Feb. 6. 2002</u>
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GIVEN NAME/FAMILY NAME <u>Chul-Soo CHO</u>	INVENTOR'S SIGNATURE <u>[Signature]</u>	DATE* <u>Feb. 6. 2002</u>
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DATE: 06/20/2002
TIME: 12:21:27

6/20/02

RAW SEQUENCE LISTING
PATENT APPLICATION: US/10/049,663

DATE: 06/20/2002
TIME: 12:21:27

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Output Set: N:\CRF3\06202002\J049663.raw

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RAW SEQUENCE LISTING

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DATE: 06/20/2002

TIME: 12:21:27

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VERIFICATION SUMMARY
PATENT APPLICATION: US/10/049,663

TIME: 12:21:28

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Rec'd PCT/PTO

22 MAY 2002

10049663.021502

#4

SEQUENCE LISTING

<110> KIM, Ho-Youn
PARK, Jong-Sang
RYOO, Zae-Young
BAE, Eui-Young
LEE, Woo-Kyoung
CHO, Chul-Soo
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KIM, Wan-Uk

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